Section A-Research paper

EVALUATION OF MICROLEAKAGE IN CLASS II CAVITIES USING DIFFERENT COMPOSITE FILLING MATERIALS AND ADHESIVE STRATEGIES UNDER CONFOCAL LASER SCANNING MICROSCOPE-AN INVITRO STUDY.

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Abstract

Aim: To evaluate microleakage in class II cavities using different composite materials and adhesive strategies under CLSM. Materials and methods: Standardized class II cavities (MO) were prepared in found sound extracted human upper premolars. The cervical margin of the proximal box was located at 1mm occlusal to the cementoenamel junction (CEJ). The prepared teeth were divided into two groups of 20 teeth each and then each group was subdivided into two subgroups of 10 teeth each. The samples of subgroup I_1 and II_1 (n=10 each) were subjected to etching process using 3M ESPE Scotchbond Universal etchant followed by bonding with the Adper Single Bond Plus Adhesive (3M ESPE, St Paul, MN, USA) bonding agent. The samples of subgroup I_2 and II_2 (n=10 each) were subjected self-etch adhesive strategy by applying Adper™ Easy Bond Self-Etch Adhesive (3M ESPE, St Paul, MN, USA). Then, all the samples in Group I were restored with Filtek™ Z350 XT Universal Restorative composite resin by using oblique increment technique with approximately 2 mm thickness.The samples in Group II were restored with Filtek™ Bulk Fill Posterior Restorative composite resin in a single increment of 4 mm thickness. All the restored teeth were stored in distilled water for 24hrs at room temperature, thermocycled and then soaked in Rhodamine B dye for 48 hrs. Teeth were then sectioned for evaluation of microleakage along the tooth-restorative interface in the occlusal and gingival regions using a CLSM. Data were collected and statistically analyzed using Kruskal-wallis one way analysis of variance (ANOVA) and Mann-Whitney U-test. Results: Statistically significant difference was observed in microleakage scores for occlusal and gingival margins between the subgroups of Group I and Group II using Kruskal - Wallis one way analysis of variance (ANOVA) test. The self-etch bonding strategy recorded a significantly more dye penetration with highest mean score (1.96 ± 0.79; $P<0.001$, 1.89 ± 0.64; $P<0.001$)) when compared to etch and rinse bonding strategy (1.54 ± 0.61; P<0.001, 1.56 ± 0.78; P<0.001)) in Group I and Group II respectively. The Mann-Whitney test was performed to evaluate significant differences of occlusal mean scores and gingival mean scores between subgroups but the results were not statistically significant within each subgroup though the microleakage mean scores were higher at gingival margins when compared to occlusal margins. Conclusion: All the test groups showed some amount of microleakage regardless of the test material or location of the margin or placement technique. Microleakage scores are higher at gingival margin compared to occlusal margin. Nevertheless, Etch and Rinse adhesive could be considered as the adhesive strategy of choice in class II situations in majority of cases as it showed low microleakge scores compared to Self-Etch adhesive strategy.

Keywords: Microleakage, class II cavity, conventional nanohybid composite, bulk fill nanohybrid composite, adhesive strategies.

Introduction

In restorative dentistry, a carious or defective tooth is restored with a restorative material to revert its normal form, function and aesthetics. Among many direct restorative materials available, silver amalgam and composite resins are widely used. The demand for an esthetic smile among people nowadays led to the development of posterior composites and have replaced silver amalgam restorative materials which were once predominantly used and became popular as a direct restorative material due to their ability to replace the tooth structure in both appearance and function[1].

There has been tremendous progress in resin composite materials since their introduction to dentistry as bisphenol a glycidyl methacrylate (Bis-GMA). Since then various formulations have been proposed by altering the functional groups which led to the development of current composites[2].

Despite the advancements of resin composite resins in recent times, clinicians still encounter the problem of polymerization shrinkage during the restorative procedure which plays a pivotal role on the marginal adaptation of resin composite restorations.Improper marginal adaptation results in gap formation between composite resin and the cavity walls that results in microleakage, which is a major concern as it contributes to restoration failure [4]. Marginal microleakage first defined by Kidd in 1976 as a process where there is clinically undetectable penetration of bacteria, their metabolites, enzymes, toxins, ions, and other cariogenic factors between the filling and the cavity wall[3]. From previous studies, it is evident that microleakage can be at micron level or at nanometer level[4]. Apart from marginal discolouration, postoperative sensitivity, pulpal irritation, and secondary caries other adverse effects of microleakage may include marginal defects which favour dental plaque accumulation leading to periodontal problems[5]. These are the most frequent reasons to replace or repair an adhesive restoration**.** Therefore, countering the polymerization shrinkage stress is one of the most challenging aspects for a clinician as the most essential factors determining the success of a restoration placed in a cavity are the marginal seal and absence of microleakage.

Many attempts have been made to decrease microleakage of adhesive restorations, such as development of new resin monomers and filler systems, increasing the filler content in its composition, flowable resin liner application, incremental filling technique, type of light source, control of curing light irradiance and changes in C-factor and direction of polymerization shrinkage. The restorative techniques that reduce the level of stress due to resin composite polymerization shrinkage have been suggested[6].However, no technique has been shown to be perfectly effective in reducing the effects of polymerization shrinkage[7].

In the present study, the role of adhesive strategy will be studied using total etch and self-etch adhesive systems to know their effect on microleakage in different type of composites. A conventional (methacrylate based) nanohybrid resin-based dental composite and a bulk-fill (methacrylate based) nanohybrid high viscosity composite were used in the study to evaluate the microleakage using different adhesive strategies. Oblique incremental technique was used for restoring the class II cavities using conventional nanohybrid composite as previous studies stated that the oblique (wedge-shaped) layering technique reduces the C-factor and limits the development of contraction forces between opposing walls and hence decreases the polymerization shrinkage stresses when compared with other layering techniques[8, 9] and Bulk fill technique was used for restoring nanohybrid Bulk fill composite.

The adhesive strategy plays an important role in preventing the deleterious effects of polymerization stress. Many developments in adhesive systems were made to overcome the problems of dentin bonding and to have more durable and predictable restorations. Different classifications were proposed to describe these systems. One of the classifications was based on the conditioning mechanism to describe the self-etch and the total etch (etch and rinse) bonding systems. Total etch and self-etch systems were further classified according to the number of steps. Total etch systems depend mainly on removing the outer layer of enamel and the smear layer produced as a result of instrumentation to ensure high bonding properties, whereas the self-etching adhesives only partially dissolves those components. The gold standard system used among the different adhesive systems is currently, considered the three-step total-etch system[10].

Studies showed the sixth generation bonding systems exhibited statisfactory bonding to dentin but not to enamel and the reason could be due to insufficient etching to enamel because of its high mineral content[11]. In addition, their availability in two bottles in which one drop of liquid from each will be mixed together before application to the tooth structure. This can probably result in errors to occur due to the use of unequal ratio of liquids mixed or if not followed manufacturers' instructions properly. So, 7th generation one bottle adhesive systems have been intoduced to overcome theses procedural errors.One of the advantages of using single-step, self-etch, one-component adhesives is it can prevent discrepancies occurring between the depth of etching and resin monomer penetration. This is because the single- step, self-etch, one-component adhesive systems form a continuous layer by simultaneous demineralization with acidic monomers, followed by resin monomer penetration into the dentin substrate[12].Although there have been reports regarding the performance of total-etch and self-etch (5th and 6th generations) adhesive systems, studies and reported data on the capability of the newly introduced self-etch, one-step, one-component adhesive system (7th generation) in sealing the margins of restorations in class II cavities is very limited. Moreover, most of the previously conducted studies on self-etch adhesives tested these materials on Class V preparations[13].

In this study, Class II cavity preparation is considered to test the in vitro performance of marginal adaptation of the composite resins as one of the reasons is gingival cavo-surface margins of Class II restorations could be a factor for an early area of failure due to its limited access of proximal boxes making the placement of the material more challenging.The other reason is the critical isthmus portion can be a challenging area for any restorative materiasl[14].

In the present study, CLSM, a nondestructive technique for visualizing subsurface tissue characteristics[15], is considered as a reliable tool to assess the microleakage at low magnification (×10). This provides more accurate detection of microleakage due to its high resolution images.

As there is no much literature available on the effect of using self-etch, one-step, one-component adhesive compared to etch and rinse adhesive systems in class II restorations on microleakage, this in vitro study was conducted to evaluate the marginal gaps qualitatively by measuring the microleakage between different type of composite materials with the tooth structure using different adhesive strategies under CLSM and the null hypothesis of the study is that different adhesive strategies will have an effect on the microleakage in class II composite restorations.

Materials and methods

Materials

The materials used in this study were as shown in Table 1.

Table 1. List of materials

S.NO.	MATERIALS	MANUFACTURER	COMPOSITION	DESCRIPTION
	Filtek™ Supreme Ultra Universal	3M ESPE	Bisphenol-A diglycidyl ether dimethacrylate, urethane dimethacrylate (UDMA), triethyleneglycol dimethacrylate (TEGDMA), and bisphenol A polyethylene glycol dietherdimethacrylate (6) resins. The filler is a combination of silica filler and zirconia filler	Conventional (Methacrylate based) nanohybrid resin-based dental composite
	Filtek™ Bulk Fill Posterior	3M ESPE	Aaromatic dimethacrylate (AUDMA), addition-fragmentation monomer (AFM),	Bulk-fill (Methacrylate based)

	Restorative		urethan dimethacrylate(UDMA), dodeconated dimethacrylate (DDDMA), Silica (20 nm), zirconia (4-11 nm), zirconia/ silica clusters, ytterbium fluoride (100 nm agglomerate particles)	Nanohybrid high viscosity composite
	Adper™ Single Bond Plus Adhesive	3M ESPE	Bis-GMA, HEMA, dimethacrylates, silica nanofiller (5 nm), polyalquenoic acid copolymer, initiators, ethanol, water	Etch-and-rinse adhesive
	Scotchbond™ Universal Etchant - Etching Gel	3M ESPE	34% phosphoric acid, water, synthetic amorphous silica, polyethylene glycol, aluminum oxide (Scotchbond Universal Etchant)	Etchant
	Adper™ Easy Bond Self-Etch Adhesive	3M ESPE	2-hydroxyethyl methacryate (HEMA) Bis-GMA Methacrylated phosphoric esters 1,6 hexanediol dimethacrylate Methacrylate functionalized Polyalkenoic acid (Vitrebond™ Copolymer) Finely dispersed bonded silica filler with 7 nm primary particle size Ethanol Water Initiators based on camphorquinone Stabilizers	Self-Etch Adhesive

Specimen preparation

A total of 40 human maxillary premolars, extracted for periodontal/orthodontic reasons were selected and were cleaned with a hand and ultrasonic scaler (Wood Pecker Medical Instrument. Co. Ltd China) from any soft tissues or hard calculus deposits, then immersed in 10% formalin for 5 days for disinfection , then finally stored in normal saline solution at room temperature and were used for the study within six months[16]. The teeth were fixed with sticky wax to the base of plastic cylinder. The cylinder was filled with modelling wax so that only root was embedded within the modelling wax.

A standardized class II mesio-occlusal cavity preparation was prepared in all teeth using coarse diamond fissure points with a high-speed hand under profuse water cooling and finished with finishing diamond points. The overall dimensions of the cavities were standardized as follows: A width of 4 mm bucco-lingually and a length of 4 mm occluso-gingivally with a depth of 2 mm axially were prepared in the cavities. The gingival margin of the proximal box was located 1mm occlusal to the cementoenenamel junction (CEJ). Periodontal probe was used to confirm dimensions. All the cavosurface margins were prepared without beveling and all internal line angles were rounded. To ensure standardization to all restorative procedures, the same degree of cure and polymerization reaction between the studied groups was achieved by using a single LED light curing unit.

Restorative procedure

The teeth were, randomly assigned into the two experimental groups (n=20) each based on the type of composite resin selected. Each group was again, divided into following 2 subgroups (n=10) according to the type of adhesive strategy used. Universal Tofflemire retainer (AISI 420 German stainless steel) with a metal matrix band of 0.05 mm (No 1001/30, Kerr Hawe SA, Bioggio, Switzerland) was applied to all cavities.

All the samples selected for Group I and II have been restored with Nanohybrid Conventional composite resins and Nanohybrid high viscosity Bulk Fill composite resins respectively.Then in Group I and II, teeth of subgroup I_1 and II_1 (n=10 each) were subjected to etching process using 3M ESPE Scotchbond Universal etchant for 20 seconds followed by rinsing with distilled water for 15 to 20 seconds and further blot dried for 20 seconds. The Adper Single Bond Plus Adhesive (3M ESPE, St Paul, MN, USA) bonding agent, was applied and light cured using LED light curing unit for 20 seconds. Teeth of subgroup I_2 and II_2 (n=10 each) were subjected self etch adhesive strategy by applying Adper™ Easy Bond Self-Etch Adhesive (3M ESPE, St Paul, MN, USA) with the help of a disposable applicator for 20 sec to all surfaces of the cavity followed by drying with mild airflow for 5 sec until the film no longer moves, indicating complete vaporization of the solvent and curing for 10 seconds with LED light.

Then all the samples in Group I were restored with Filtek™ Z350 XT Universal Restorative composite resin by using oblique increment technique with approximately 2 mm thickness. Each increment was gently condensed with clean Teflon coated composite condenser in order to ensure complete adaptation to the underlying resin and tooth structure. The samples in Group II were restored with Filtek™ Bulk Fill Posterior Restorative composite resin in a single increment of 4 mm thickness. The occlusal anatomy was carved as exactly as possible avoiding overhangs. The 2-mm increments are cured for 20s while the 4-mm increment is light-cured for 40 s with a LED light curing unit (LED.D, Woodpecker) with output irradiance of approximately 800 mW/cm2 held in contact with the cavosurface of the tooth[7]. After removal of the matrix band, the restoration was light-cured from their buccal and lingual aspects for an additional 20 seconds on mesial side to ensure complete polymerization, followed by finishing and polishing using finishing discs and polishing pastes. All the restored samples were stored in distilled water for 24 hours before testing to ensure a complete polymerization process.

Thermocycling

All samples are subjected to thermocycling where they were alternately immersed in 5^oC to 60^oC water bathes for 1000cycles with a dwell time of 30 seconds[9]. Thermocycling was done to mimic intra-oral temperature variations. Then, the specimens were dried, and two layers of nail polish was applied except on the resin composite restoration and 1 mm area around it, and the apex was sealed with sticky wax, to avoid any dye penetration from invisible cracks, areas devoid of enamel or cementum. The teeth were then immersed in Rhodamine-B dye for 48hrs hours[1].

Microleakage analysis using CLSM:

Microleakage was evaluated using confocal laser scanning microscope at a magnification of 10×. Based on the amount of dye penetration, the microleakage scores are recorded using scoring method given by Radhika et al[1].

The scores are as under:

Scoring for dye penetration for marginal microleakage on the occlusal wall

- 0 - No dye penetration.
- 1 - Dye penetration upto half of the occlusal wall
- 2 - Dye penetration upto more than half of the occlusal wall.
- 3 - Dye penetration upto the pulpal floor.

Scoring for dye penetration for marginal microleakage on the gingival margin

- 0 - No dye penetration.
- 1 - Dye penetration upto half of the Gingival seat
- 2 - Dye penetration upto more than half or complete extension of the Gingival seat
- 3 - Dye penetration upto complete gingival seat and extended to axial walls towards the pulp.

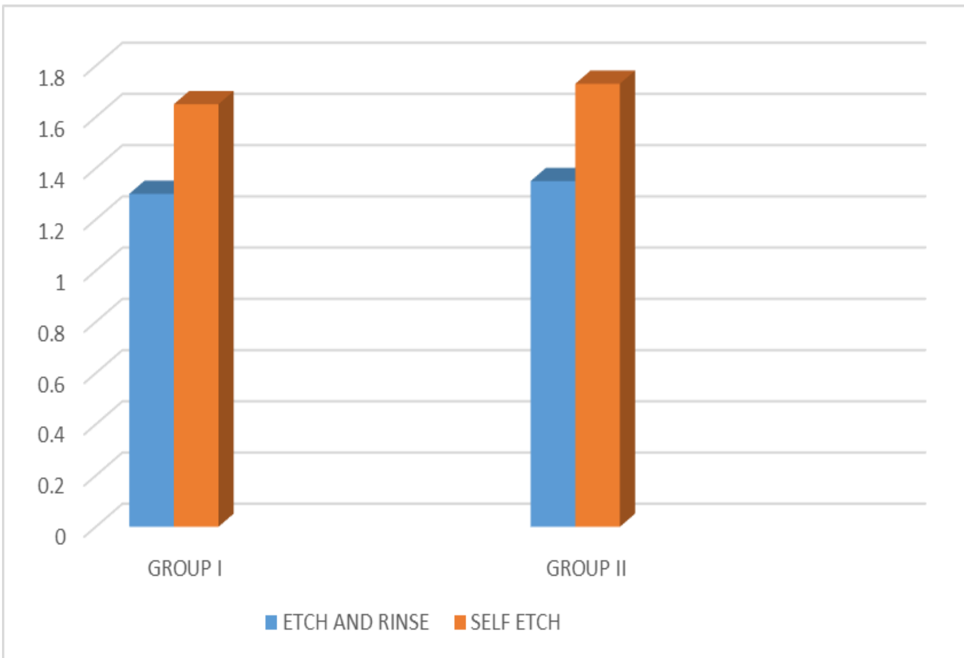
Statistical Analysis

The results of microleakage were subjected to statistical analysis using Statistical Package for Social Sciences (SPSS) version 20.0. The mean and standard deviation of microleakage scores of the four subgroups were compared using the Kruskal-wallis one way analysis of variance (ANOVA). A comparison of the occlusal and gingival margins of the groups was performed using Mann-Whitney U-test. $P < 0.05$ will be considered to be statistically significant.

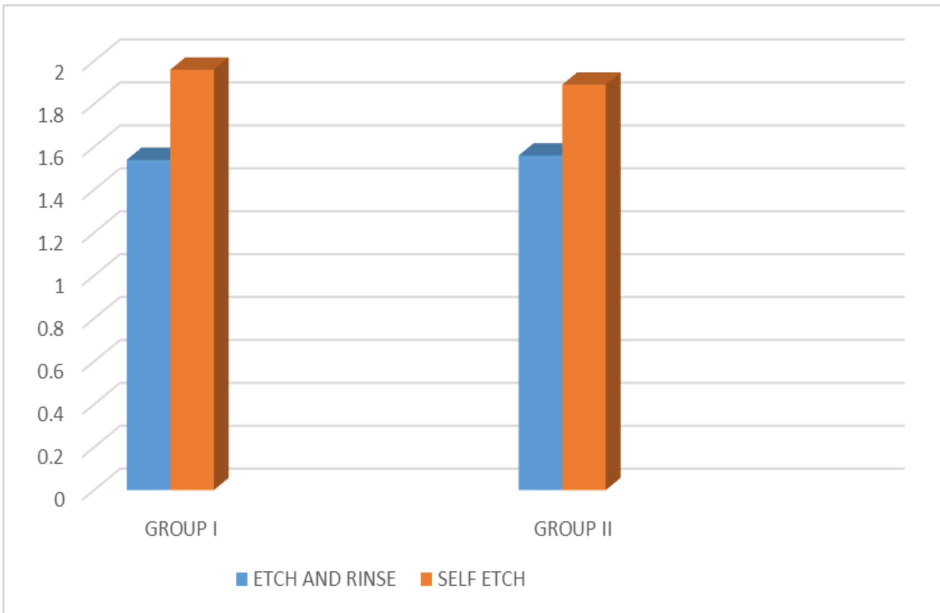
Results

All the test groups showed some amount of microleakage regardless of the test material or location of the margin or placement technique. Kruskal - Wallis one way analysis of variance (ANOVA) test displayed a significant difference in microleakage scores for occlusal and gingival margins in all the subgroups of both Group I and II .The self-etch bonding strategy recorded a significantly more dye penetration with highest mean score (1.96 ± 0.79; $P<0.001$, 1.89 ± 0.64; $P<0.001$)) when compared to etch and rinse bonding strategy (1.54 ± 0.61; $P<0.001$, 1.56 ± 0.78; $P<0.001$)) in Group I and Group II respectively as shown in Table 2&3, Graphs 1&2 .The Mann-Whitney test was performed to evaluate significant differences of occlusal mean scores and gingival mean scores between subgroups as shown in Table 4&5, Graphs 3,4,5&6 but the results were not statistically significant within each subgroup though the microleakage scores were higher at gingival margins when compared to occlusal margins.

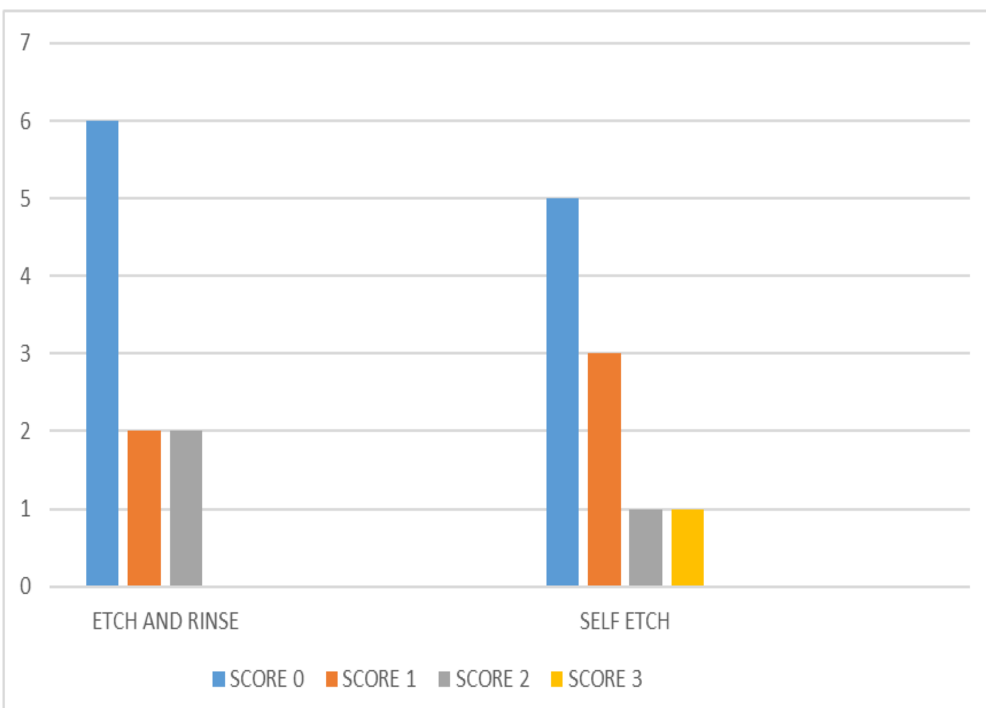
GRAPH 1. MEAN MICROLEAKGE AT OCCLUSAL MARGINS IN GROUP I AND GROUP II



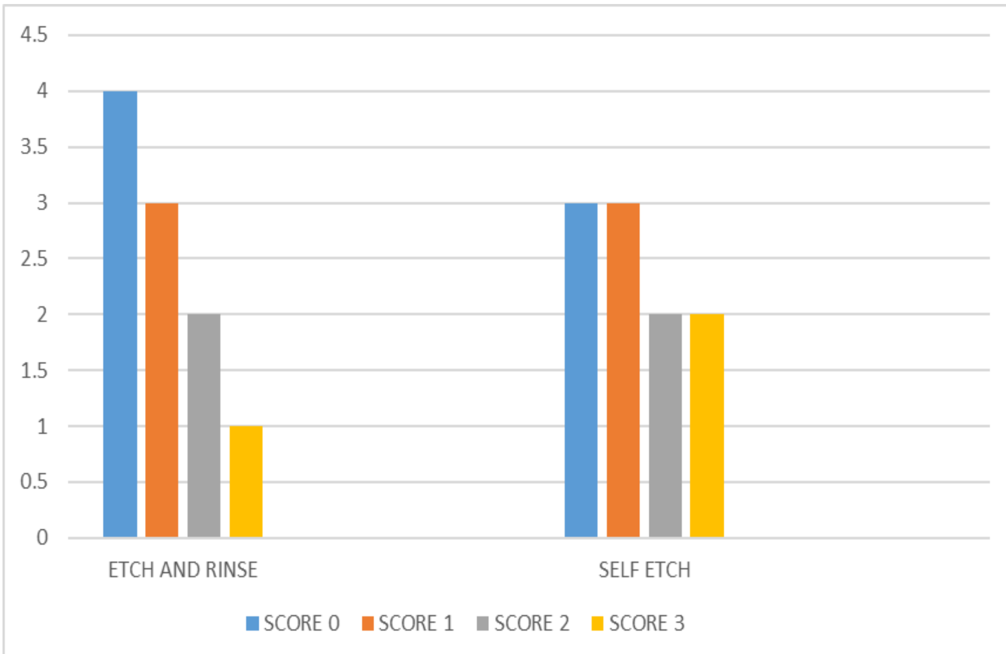
GRAPH 2. MEAN MICROLEAKGE AT GINGIVAL MARGINS IN GROUP I AND GROUP II



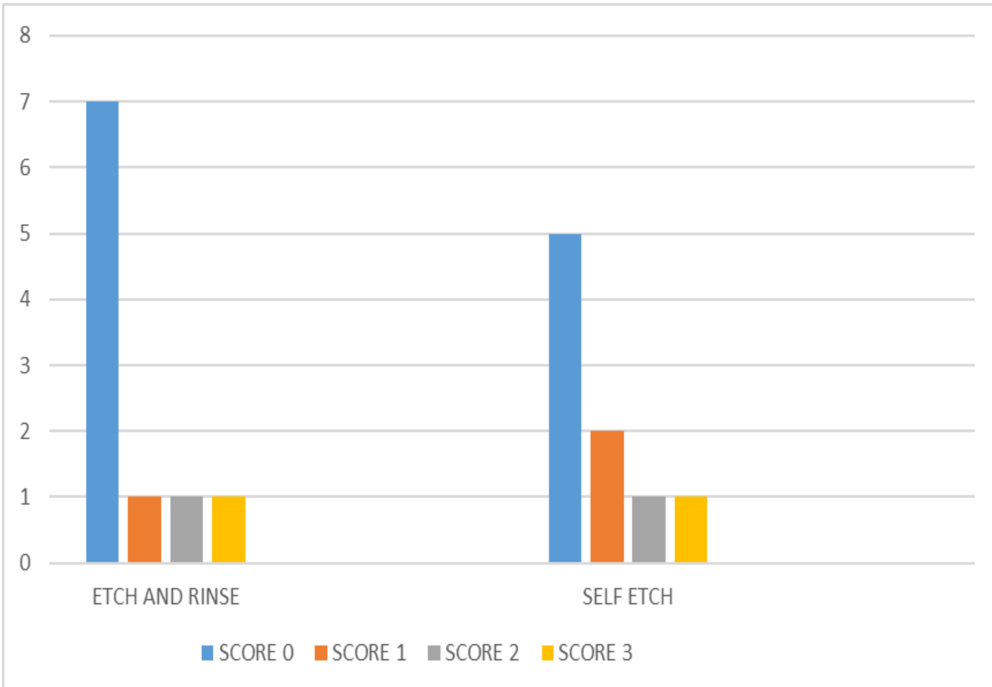
GRAPH 3. MICROLEAKGE SCORES AT OCCLUSAL MARGINS IN GROUP I



GRAPH 4. MICROLEAKGE SCORES AT GINGIVAL MARGINS IN GROUP I



GRAPH 5. MICROLEAKGE SCORES AT OCCLUSAL MARGINS IN GROUP II



GRAPH 6. MICROLEAKGE SCORES AT GINGIVAL MARGINS IN GROUP II

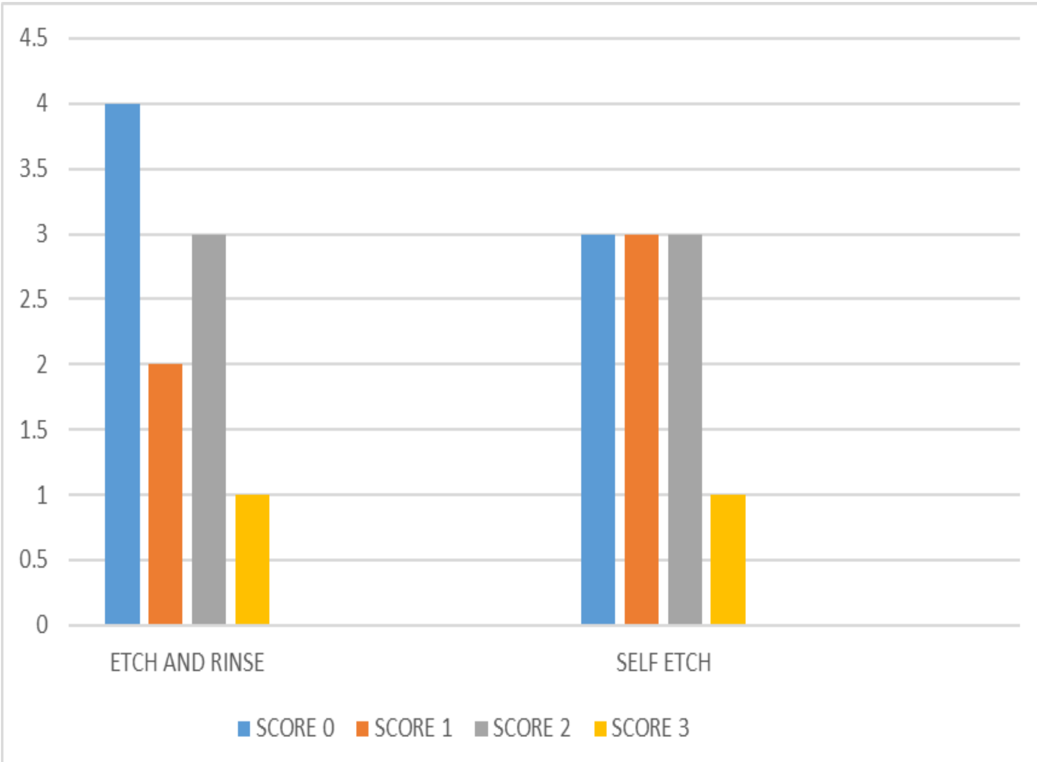


Table 2. Comparison of mean microleakage at occlusal margins using Kruskal-Wallis one way analysis of variance (ANOVA) in Group I and Group II

SUBGROUP	N	SCORE				MEAN±SD	P VALUE
		0	**1**	**2**	**3**		
SUB GROUP I_1 (ETCH AND RINSE)	10	6	2	2	0	1.30±0.72	0.001*
SUB GROUP I_2 (SELF ETCH)	10	5	3	1	1	1.65±0.79	
SUB GROUP II_1 (ETCH AND RINSE)	10	7	1	1	1	1.35±0.81	0.001*
SUB GROUP II_2 (SELF ETCH)	10	5	2	1	1	1.73±0.91	

*Statistical significance set at 0.05; **N:** Number of samples; **SD:** Standard deviation

Table 3. Comparison of mean microleakage at gingival margins using Kruskal-Wallis one way analysis of variance (ANOVA) in Group I and Group II

SUBGROUP	N	SCORE				MEAN±SD	P VALUE
		0	1	2	3		
SUB GROUP I_1 (ETCH AND RINSE)	10	4	3	2	1	1.54±0.61	0.001*
SUB GROUP I_2 (SELF ETCH)	10	3	3	2	2	1.96±0.79	
SUB GROUP II_1 (ETCH AND RINSE)	10	4	2	3	1	1.56±0.78	0.001*
SUB GROUP II_2 (SELF ETCH)	10	3	3	3	1	1.89±0.64	

*Statistical significance set at 0.05; **N:** Number of samples; **SD:** Standard deviation

Table 4. Comparison of Mean microleakage at occlusal and gingival margins using Mann-Whitney test in Group I

SUBGROUP	REGION	N	MEAN±SD	P VALUE
SUB GROUP I_1 (ETCH AND RINSE)	OCCLUSAL	10	1.30±0.72	0.109
	GINGIVAL	10	1.54±0.61	
SUB GROUP I_2 (SELF ETCH)	OCCLUSAL	10	1.65±0.79	0.142
	GINGIVAL	10	1.96±0.79	

*Statistical significance set at 0.05; N: Number of samples; SD: Standard deviation

Table 5. Comparison of Mean microleakage at occlusal and gingival margins using Mann-Whitney test in Group II

SUBGROUP	REGION	N	MEAN±SD	P VALUE
SUB GROUP II_1 (ETCH AND RINSE)	OCCLUSAL	10	1.35±0.81	0.264
	GINGIVAL	10	1.56±0.78	
SUB GROUP II_2 (SELF ETCH)	OCCLUSAL	10	1.73±0.91	0.455
	GINGIVAL	10	1.89±0.64	

*Statistical significance set at 0.05; **N:** Number of samples; **SD:** Standard deviation

Discussion

Microleakage, being a seed Sower for recurrent caries, is considered as one of the major factors which influences the success of a restoration. Studies proved that with impervious sealing of the cavity margins, the microleakage occurring at the interface can be minimized and this can be achieved by many factors. One of the factors is using an appropriate adhesive strategy to overcome the detrimental effects of microleakage. Good adhesion between the composite resin and dental tissues results in reduced microleakage[17]. Adhesives in dentistry has been evolving at a rapid rate since their introduction decades ago. Extensive research coupled with the widespread demand for dental adhesives has broadened their range of application. New approaches to bonding restorative materials to tooth substrate without phosphoric acid etching, such as self-etching systems, have recently been introduced. These simplified systems aim to reduce the technique sensitivity by reducing the number of clinical steps involved. As a result of this, their popularity is increasing.

Therefore, this study was performed to assess the microleakage of class II cavity restored with Conventional composites and bulk-fill composite resin material using different adhesive strategies under CLSM.

Class II composite resin restoration has been chosen for this study as Class II cavities are often a concern for the clinician due to limited access, difficulty in isolation, difficult and unpredictable bonding to dentin and cementum along with material limitations of polymerization shrinkage and subsequent microleakage[27]. To simulate the oral conditions, the samples were subjected to thermocycling.

Microleakage can be evaluated using several techniques. But recently a non-destructive technique like confocal laser scanning microscopy which helps visualizing the subsurface tissue properties has been popularly used to assess microleakage. In this study microleakage was measured using CLSM at 10x magnification[19].

The outcomes of the study showed that there will be some amount of microleakage irrespective of the bonding technique used. These findings could be attributed to the polymerization shrinkage which is considered an inherent property responsible for the main shortcoming of composite resin material[20]. In addition, polymerization shrinkage increases in a cavity with high a C-factor as in the case of class II cavities[1].

The hypothesis stating that there will be significant difference in microleakage among adhesive strategies using conventional nanohybrid composites and bulk fill composites have been supported by the results of this study. The results have shown significantly less microleakage with Etch and Rinse adhesives compared to self-etch adhesives in both conventional and bulk fill composites and the results are in accordance with other studies conducted[21, 22, 23]. This could be attributed to the total etch technique uses 37% phosphoric acid, which is a strong acid, to remove the smear layer on the enamel and dissolve the enamel prism, thus enabling infiltration of the surface by adhesive material. The smear layer can affect marginal adaptation of a composite resin[24].Whereas, these self-etch, seventh-generation adhesives, also called all-in-one adhesives use phosphoric acid ester monomers, a weak acid, as an etching agent. All-in-one adhesives act as semi permeable membranes, consequential a hydrolytic degradation of the resin-dentin interface. As the etching in the enamel is not as deep as etching using phosphoric acid, the resulting bond strength is weaker than that obtained using the total etch technique[25,26]. Furthermore, the smear layer that forms during cavity preparation does not dissolve thoroughly, which results in a limited resin tag that performed by self etch adhesive system[27].

There is significantly higher microleakage at gingival margins when compared to occlusal margins and the reason for this may be attributed to the presence of more organic content, configuration of the tubules and low surface energy of the dentin makes bonding relatively difficult compared to enamel[28, 29]. Another factor water sorption and stress relaxation cannot compensate the extent of shrinkage caused by polymerization [30].

However, the findings in the present study contrasted to few studies done by other researchers comprising the different adhesive techniques (Etch and Rinse versus Self Etch) with different resin based composite systems (conventional versus bulk fill composites). Some studies found no statistically significant difference among different adhesive techniques used with composite resins on microleakage[31,32] whereas other investigators reported better results with the Self Etch technique compared to Etch and Rinse technique[33,34,35].The results could be varying due to different experimental conditions like different materials, cavity preparations and placement techniques used.

Conclusion

Within the limitations of the study, it was concluded that no adhesive strategy completely eliminated the microleakage especially from gingival margins of class II cavities in both groups. Higher microleakage scores were observed at gingival margin compared to occlusal margin. Nevertheless, Etch and Rinse adhesive could be considered as he adhesive strategy of choice in class II situations in majority of the clinical scenarios as Etch and Rise adhesive strategy showed low microleakage scores compared to Self Etch adhesive strategy. Confocal Laser Sanning Microscopy is a reliable method for microleakage evaluation both qualitatively and quantitatively. Further in vivo studies are recommended for more understanding of these adhesive strategies in clinical scenarios as it is not possible to simulate the exact oral conditions in vitro , which in turn could lead to certain unavoidable bias.

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